

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Coleman *et al.*
Title: PHARMACEUTICAL USES OF
BIOPHOSPHONATES
Appl. No.: 10/578,290
International Filing Date: 12/2/2004
371(c) Date: 1/11/2007
Examiner: Marcos L. Sznaidman
Art Unit: 1612
Confirmation Number: 2763

DECLARATION OF INGUNN HOLEN UNDER 37 C.F.R. § 1.132

Dear Examiner Sznaidman:

I, DR. INGUNN HOLEN, state and declare that:

1. I am a Reader in Bone Oncology at the University of Sheffield, UK. I have a Ph.D. in Molecular Cell Biology. I have carried out research in oncology for 18 years and am skilled in the study of anti-tumor compounds, including the study of tumor cell growth inhibition and induction of cancer cell apoptosis of biosphosphonate compounds. I am a co-inventor of the invention recited in claims 7, 11, 26, and 28 of the patent application identified above. I have reviewed the Office Actions dated January 6, 2009 and June 17, 2009, pertaining to the above-identified patent application and the reference cited by the Office (Jagdev *et. al.*, *British J. of Cancer*, 84:1126-1134, 2001).

2. Jagdev *et al.* exposed breast cancer cells to a combination of ZOL (2-imidazol-1-yl-1-hydroxyethane-1,l-diphosphonic acid, also known as Zoledronic acid) and PAC (Paclitaxel) for a period of 72 hours to induce apoptosis of the breast cancer cells. (*Id.*, p. 1130.)

The time period for treatment ZOL as used in the cited reference and therefore the concentrations of ZOL are not clinically relevant because they would be too high for use in humans. It is not possible to clinically achieve the stated concentration of ZOL in humans for the stated period of time for the following reasons:

(a) due to well-known renal toxicity, acute phase reactions, and osteonecrosis of the jaw, ZOL is typically administered for cancer treatment in small carefully controlled doses widely spaced in time: e.g., by IV infusion for 15 minutes at a dose of about 4 mg/patient no more often than once every 3-4 weeks (Diel *et al.* "Adverse Effects of Bisphosphonates," *J. Support. Oncol.* 5: 475-82, 2007);

(b) following the standard 15 minute IV infusion, ZOL remains in the plasma for no more than a few hours before being excreted or localizing to the bone (Chen *et al.*, "Pharmacokinetics and pharmacodynamics of zoledronic acid in cancer patients with bone metastases," *J. Clin. Pharmacol.* 42:1228-36, 2002 (p. 1229, 1st col.; p. 1230 – section titled "Blood and Urine Sampling,"; Fig. 4; p. 1235, 1st col.); see also Diel *et al.* at p. 476, Table 2 (stating that the half life of zoledronic acid is 1.4-1.9 h));

(c) therefore, I believe that repeated and prolonged infusion periods of zoledronic acid would be required to reproduce the 72 hours exposure tested in the Jagdev reference and that this is not achievable in clinical practice.

3. While employed with University of Sheffield, I performed or caused to be performed the sequential administration of the compounds, ZOL and PAC, set forth in Experiments 1 and 2 of U.S. Patent Application No. 10/578,290, pp. 15-16 as filed. Experiment 1 showed proof of principle by demonstrating that sequential administration of 2 nM PAC (4 hours) followed by 25 uM ZOL (1 hour) to breast cancer cells (MCF7 cells) produced a

synergistic response (6.1% apoptosis) compared to PAC or ZOL alone. Experiment 2 showed a similarly synergistic response upon incubation of the MCF7 cells with 2 nM PAC for 4 hours followed by 1 μ M ZOL for 1 hour (4.1% apoptosis) compared to the same concentrations of PAC and ZOL alone (1.25% and 0.25% apoptosis). The exposure time of the cells to ZOL in both experiments was only 1.3% as long as the exposure time reported in Jagdev *et al.* These results were also reported in the paper Neville-Webbe, H.L., *et al.*, *Tumor Biol.*, 27:92-103, 2006, on which I am a coauthor (see pp. 96-98, Figs. 3 and 4). The latter paper reports that the same synergistic effect was achieved in another breast cancer cell line (MDA-MB-436) as shown in Fig. 4b.

4. The concentrations and exposure periods of PAC and ZOL set forth in Experiment 2 of the application are clinically relevant. *Id.*, p. 96. In particular, the concentration of ZOL (1 μ M) is quite similar to the peak plasma concentration of ZOL, which is 1-2 μ M, following a 4mg IV infusion before it is excreted or localizes to bone. *Id.* The 1 hour incubation period of the cells with ZOL also tracks more closely the *in vivo* exposure time of cancer cells in a patient to ZOL before the ZOL is excreted or localizes to bone. Thus, in contrast to the cited prior-art, the amount of ZOL used and the shorter incubation period described in the current patent application is clinically achievable. *Id.* (See also Wilson, W.H., *et al.*, *J. Clin. Oncol.* 12:1621-29, 1994.) Likewise, due to the shorter exposure time, the 4 hour incubation with PAC in our experiments is more clinically relevant to the treatment of breast cancer than the 72 hour period described in Jagdev *et al.*

5. As one of skill in the art, before performing these experiments, I could not have predicted whether the shorter incubation periods used for both ZOL and PAC (~ 1.3% and 5.6% as long, respectively) would provide synergistic, additive or no therapeutic effect, in view

of Jagdev's disclosure. The large synergistic effect observed at such a low concentration and short time was surprising to me.

6. I hereby acknowledge that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. § 1001) and may jeopardize the validity of the above-referenced application or any patent issuing thereon. All statements made of declarant's own knowledge are true and all statements made on information and belief are believed to be true.



INGUNN HOLEN, Ph.D.

November 16, 2009
Date